

Molecular and Genetic Analysis of the Yeast Early Meiotic Recombination Genes *REC102* and *REC107/MER2*

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By selecting for mutations which could rescue the meiotic lethality of a *rad52 spo13* strain, we isolated several new Rec genes required relatively early in the meiotic recombination process. This paper presents data to confirm that two of them, *REC102* and *REC107*, are general, meiosis-specific recombination genes that have no detectable role during mitosis. Sequence analysis and genetic complementation indicate that *REC107* is identical to the *MER2* gene. No sequences related to *REC102* have been found in the GenBank or EMBL collections. *REC102* is expressed only in meiosis, prior to the reductional division, at about the time that genetic recombination occurs. Examination of the *REC102* sequence indicates the presence of several sequences which may play a role in the regulation of its expression; however, the URS1 sequence commonly found in genes expressed early in meiosis is not present.

High levels of genetic recombination, homologous chromosome pairing, synaptonemal complex formation, and reductional division are unique events that occur during meiosis. These events must take place for meiosis to occur properly (2). With very few exceptions (e.g., *Drosophila* males [5]), the failure of chromosomes to recombine leads to random segregation during the reductional division, resulting in aneuploid and inviable meiotic products. Thus, the process of genetic recombination is one of the essential steps in the developmental pathway of meiosis.

At the chromosomal level, all the events of meiosis in *Saccharomyces cerevisiae* occur as expected for a typical eucaryote. During the last several years, a large number of genes required for events specific for yeast meiosis have been defined. For example, the *SPO12* and *SPO13* genes are essential for the reductional division (19). Cells containing mutations in either gene produce two diploid spores that are primarily the products of a single equational division (19). More recently, mutations in the *RED1* gene have been reported both to affect the reductional division and to reduce meiotic recombination (37). The *HOP1* gene is required for synaptonemal complex formation (16, 17). Genes that are required for the normal level of meiotic recombination include *RAD50* (13, 24), *RAD52* (22, 24), *RAD57* (13), *SPO11* (1, 20), *MER1* (9, 10), *MEI4* (27), and *MER2* (8). Mutations in any of these genes reduce meiotic recombination and result in very poor spore viability.

In an attempt to define new genes required for the initiation of meiotic recombination, we devised a selection which allowed only early Rec[−] mutants to survive after meiosis (23). In the presence of a *spo13* mutation, recombination is not essential for the production of viable spores, presumably because no reductional division occurs (24). However, only some Rec[−] mutants produce viable spores after meiosis when the *spo13* mutation is present. For example, *rad50* mutations are rescued by *spo13*, but *rad52* mutations are not (24). The best current hypothesis is that mutants blocking recombination early (before strand exchange) are rescued, whereas mutants blocking recombination later generate intermediates that prevent even an equational segregation (22,

23). Using this approach, we isolated 177 putative early meiotic recombination mutants and examined 56 of them (23). This analysis resulted in the isolation of five new Rec genes (*REC102*, *REC104*, *REC107*, *REC113*, and *REC114*); mutations in all of these appeared to confer the expected early meiotic Rec[−] mutant phenotype. Initial analysis of strains containing the *rec102* or *rec107* mutation demonstrated no increase in meiotic recombination over the background mitotic levels at the two marker loci examined. Neither mutant appeared to have defects in mitotic growth rate or mitotic recombination (also measured at two loci) (23).

We report a detailed characterization of the recombination phenotypes of mutations in two genes, *REC102* and *REC107*, including data from a null mutation of *REC102*. We also analyze the sequence of *REC102* and its expression patterns. All the data are consistent with the hypothesis that the *REC102* gene encodes a protein required for early meiotic recombination events. In the process of restriction mapping and sequencing *REC107*, we found that it was identical to the *MER2* gene identified by Engebrecht et al. (8).

MATERIALS AND METHODS

Strains, media, and plasmids. The yeast strains used in this study are listed in Table 1. *S. cerevisiae* strains were cultivated in YPD medium or C-URA (synthetic complete medium with all auxotrophic nutrients except uracil) (34). YPA and sporulation medium are described by Malone et al. (23). *Escherichia coli* RK1448 was used for amplification of plasmids (22). The media and the yeast transformation protocol are described by Hoekstra and Malone (15). The *cdc25-5* strain was a gift from Kelly Tatchell (North Carolina State University).

The plasmids pRS306 and pRS316 were kindly provided by P. Hieter (36). Subclones were constructed by the method of Sambrook et al. (32). The GC8 vector was supplied by Jan Fassler (University of Iowa) and contained a 7-kb *HindIII* fragment including the *PYK1* gene inserted into pBR322. The plasmid pCM208-2I was obtained by *ExoIII* deletion between bp +538 of the *REC102* gene and the *SstI* site of pCM208. A null mutation of *REC102* was constructed by inserting the 2.3-kb *BspEI*-*BamHI* fragment containing

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TABLE 1. Yeast strains and plasmids

Strain or plasmid	Relevant genotype	Reference or source
Strains		
C4-1	<i>MATa rec102-1 can1 ura3-52 + cyh2 trp5-c leu1-c</i> <i>MATa rec102-1 CAN1 ura3-52 hom3 CYH2 + +</i>	23
K65-3D	<i>HO MATa</i> <i>HO MATa</i>	23
C3-16	<i>MATa spo13-1 lys2-2 CAN1 ura3-1 met13-c cyh2 trp5-c leu1-c</i> <i>MATa spo13-1 lys2-1 can1 ura3-52 met13-d CYH2 trp5-2 leu1-12</i>	23
C3-15	<i>MATa rec102-1 lys2-2 CAN1 ura3-1 met13-c cyh2 trp5-c leu1-c</i> <i>MATa rec102-1 lys2-1 can1 ura3-52 met13-d CYH2 trp5-2 leu1-12</i>	This paper
C3-13	<i>MATa rec102-1 spo13-1 lys2-2 CAN1 ura3-1 cyh2 trp5-c leu1-c</i> <i>MATa rec102-1 spo13-1 lys2-1 can1 ura3-52 CYH2 trp5-2 leu1-12</i>	23
C3-14	<i>MATa rec102-1 spo13-1 lys2-2 CAN1 ura3-1 met13-c cyh2 trp5-c leu1-c</i> <i>MATa rec102-1 spo13-1 lys2-1 can1 ura3-52 met13-d CYH2 trp5-2 leu1-12</i>	23
C2-1	<i>MATa rec107-1 + tyr1-1 can1 ura3-13 hom3 CYH2</i> <i>MATa rec107-1 lys2-1 + CAN1 ura3-13 + cyh2</i>	23
RM162	<i>MATa rec107-1 spo13-1 lys2-2 tyr1-2 his7-1 CAN1 ura3-1 met13-c cyh2 leu1-c</i> <i>MATa rec107-1 spo13-1 lys2-1 tyr1-1 his7-2 can1 ura3-13 met13-d CYH2 leu1-12</i>	23
C7-2	<i>MATa rec102::URA3 spo13-1 lys2-2 CAN1 cyh2 trp5-c leu1-c</i> <i>MATa rec102::URA3 spo13-1 lys2-1 can1 CYH2 trp5-2 leu1-12</i>	This paper
C7-4	<i>MATa rec102::URA3 lys2-1 can1</i> <i>MATa rec102::URA3 lys2-2 CAN1</i>	This paper
C7-5	<i>MATa lys2-2 CAN1 met13-c cyh2 trp5-c leu1-c</i> <i>MATa lys2-1 can1 met13-d CYH2 trp5-2 leu1-12</i>	This paper
Plasmids		
pRS306	<i>URA3 Amp^r</i>	36
pRS316	<i>CEN6 ARSH4 URA3 Amp^r</i>	36
pCM201	YCp50 + <i>REC102</i> (7-kb original clone)	This paper
pCM208	pRS316 + <i>REC102</i> (<i>EcoRI-SpeI</i> 4.6-kb fragment)	This paper
pCM209	pRS316 + <i>REC102</i> (<i>EcoRI-SpeI</i> 4.6-kb fragment)	This paper
pCM208-2I	pCM208 (without the fragment between +538 of the <i>REC102</i> gene and the <i>SstI</i> site)	This paper
pCM701	YCp50 + <i>REC107</i> (15-kb original clone)	This paper
pCM706	pRS316 + <i>REC107</i> (<i>EcoRI-ClaI</i> 4.4-kb fragment)	This paper
pSM22	pBR322 + <i>rad52::URA3</i>	26
GC8	pBR322 + <i>PYK1</i>	J. Fassler
pCM210	pUC19 + <i>REC102</i> (<i>BspEI-BamHI</i> 2.3-kb fragment)	This paper
pCM211	pCM210 with <i>EcoRV</i> 0.7-kb fragment of <i>REC102</i> removed and 1.1-kb <i>URA3</i> fragment inserted	This paper

REC102 into pUC19 (pCM210); the 0.7-kb *EcoRV* fragment from -280 to +440 was then removed, and the 1.1-kb *URA3* fragment was inserted (pCM211). This leaves only 53 amino acids of the original protein and removes most of the promoter region. The *BspEI-BamHI* fragment from pCM211 was used to transform yeast cells to create the null mutant.

Yeast genetics methods. Mating, diploid isolation, and tetrad analysis were carried out by standard procedures (34). Yeast transformation with lithium salts was performed by the method of Ito et al. (18). The spheroplast method was used to transform integrating vectors (14). The *rec102 spo13 rad52* diploid strain was obtained by transforming *rec102 spo13* haploids with a 3.4-kb *BamHI* linear fragment containing a *rad52::URA3* disruption (26).

Cloning and analysis of *REC102* and *REC107*. *REC102* and *REC107* genes were cloned as described by Malone et al. (23). A 4.4-kb *EcoRI-ClaI* fragment from pCM701 was inserted into pRS316, creating pCM706, which was used to sequence *REC107*. For the sequencing of *REC102*, pCM208 was constructed by inserting a 4.6-kb *EcoRI-SpeI* fragment from pCM201 into pRS316. pCM209 is pRS316 containing the same *EcoRI-SpeI* fragment as pCM208 but in the opposite orientation. This reverse orientation was constructed by treating the *SpeI* end of the 4.6-kb *EcoRI-SpeI* fragment with *S1* nuclease (creating a fragment with one *EcoRI* end and one blunt end). This fragment was inserted into pRS316 at

the *HindIII* (treated with *S1* nuclease) and *EcoRI* sites. Fragments for DNA sequencing were generated from pCM208 and pCM209 by using the Erase-a-Base kit (Promega). Dideoxy sequencing was performed as described previously (33) with the Sequenase kit (U.S. Biochemicals). The search for homology of DNA and protein sequences was performed with GenBank and EMBL data bases by using Wordsearch and Fasta programs (6).

Regulation of expression of *REC102*. RNAs from mitotic and meiotic cells were extracted as described by Elder et al. (7). Northern (RNA) blot analysis was performed as described by Sambrook et al. (32). The *PYK1* probe was made by the random priming method (Bethesda Research Laboratories) with the 0.66-kb *KpnI* fragment of plasmid GC8. *PvuI*-digested pCM208-2I was used as a template for T7 polymerase to transcribe an antisense RNA probe of *REC102* (Stratagene). This probe extends from positions +535 to +10 (see Fig. 5). The amount (area of band) of each RNA from the Northern blot was measured by a video densitometer model 620 (Bio-Rad). To plot the relative amount of *REC102* mRNA during meiosis, the ratio of *REC102* mRNA to *PYK1* mRNA and also the ratio of the 3.39-kb rRNA to the 1.79-kb rRNA were calculated. These values were then normalized to the highest ratio, which was given a value of 1.

Nucleotide sequence accession number. The nucleotide

sequence data reported in this paper have been submitted to GenBank and assigned the accession number M74045.

RESULTS

Mitotic recombination and repair. To verify the hypothesis that the *REC102* and *REC107* genes are meiosis-specific recombination (Rec) genes, the frequency of spontaneous mitotic recombination was measured at a number of different loci on several chromosomes. (Our initial experiments examined only two loci, and to verify the hypothesis that these mutations did not affect mitotic exchange, we examined more loci.) To monitor mitotic gene conversion, we examined the frequency of prototroph formation at heteroallelic loci. The frequency of drug-resistant colonies, produced from diploids heterozygous at two loci for a recessive drug resistance marker, was used to calculate mitotic crossing-over. (The frequency of drug-resistant colonies is the sum of crossing-over, gene conversion, and chromosome loss; however, under normal conditions, both gene conversion and chromosome loss occur at frequencies several orders of magnitude lower than crossing-over [30].) The data in Table 2 demonstrate that the *rec102-1*, *rec102::URA3*, and *rec107-1* mutations do not have any significant effect on mitotic recombination. We detect no significant differences between the effects of the *rec102-1* mutation and the null mutation. (Although several of the diploids examined contain the *spo13* mutation, it has no effect on the frequency of mitotic recombination [19].) We conclude that *REC102* and *REC107* are not required for mitotic recombination, consistent with their proposed roles as meiosis-specific recombination genes. Also in agreement with this hypothesis is the observation that mutations in either gene did not confer defects in DNA repair. For example, no sensitivity to UV (Fig. 1) or to the DNA-damaging agent methyl methanesulfonate (Fig. 2) is apparent.

Rescue of *rad52 spo13* diploids. Although the *rec* mutants were isolated by selecting for meiotic survival of a *rad52 spo13* cell, the strain used for the selection was a haploid so as to maximize the recovery of recessive mutations (23). It was formally possible that the *rec102* mutations would not rescue a *rad52 spo13* diploid strain. To eliminate this possibility, we examined a *rec102 rad52 spo13* diploid and found that it sporulated as well as *Rec⁺ spo13* cells and produced 78% viable spores (data not shown). We conclude that a *rec102* mutation provides an early recombination block that rescues *rad52* mutants in diploids as well as in haploids.

Meiotic recombination. The history of *Rec⁻* mutations includes a number of cases in which the putative *Rec⁻* mutation affected recombination only at one or a limited number of loci (e.g., references 12 and 31). To determine whether the *rec102* or *rec107* mutation caused a general deficiency in meiotic recombination, *rec spo13* strains were first examined for the frequency of prototroph formation at several different heteroallelic loci on three different chromosomes (Table 3). Heteroallelic recombination is a measure of gene conversion (26, 29, 31). The wild-type control exhibited a typical 1,000-fold increase in gene conversion after meiosis (Table 3). However, the data indicate that mutations in either *REC102* or *REC107* completely eliminate the increase in gene conversion normally found after meiosis (Table 3). The frequency of drug-resistant colonies in a *spo13* meiosis is a measure of gene conversion, crossing-over between the resistant locus and the centromere, and aberrant segregation; crossing-over appears to be the predominant contributor (22). Therefore, data in Table 3 suggest that both *rec102*

TABLE 2. Spontaneous mitotic recombination in *Rec⁻* and *Rec⁺* diploids^a

Strain	Genotype	Prototroph frequency (10 ⁵)					Drug-resistant colony frequency (10 ⁴)		
		<i>ura3-1</i> <i>ura3-52</i>	<i>trp5-c</i> <i>trp5-2</i>	<i>leu1-c</i> <i>leu1-12</i>	<i>met13-c</i> <i>met1-3d</i>	<i>his7-1</i> <i>his7-2</i>	<i>pyr1-2</i> <i>pyr1-1</i>	<i>lys2-2</i> <i>lys2-1</i>	<i>can1</i> <i>CAN1</i>
C3-16	<i>REC spo13-1</i>	11 ± 69 ^b	5.2 ± 38 ^b	4.3 ± 4.0 ^c	1.9 ± 0.2	0.36 ^d	0.30 ^d	0.15 ± 0.07	5.2 ± 3.1
	<i>REC spo13-1</i>								4.7 ± 0.7 ^c
C3-14	<i>rec102-1 spo13-1</i>	7.7 ± 3.5 (0.7)	2.2 ± 2.0 (0.42)	4.7 ± 2.6 ^c (1.1)	2.7 ± 1.9 (1.4)	ND	ND	0.15 ± 0.10 (1.0)	8.4 ± 6.3 (1.6)
	<i>rec102-1 spo13-1</i>								9.0 ± 3.4 ^c (1.9)
C7-2	<i>rec102::URA3 spo13-1</i>	ND	1.4 ± 1.6 (0.27)	2.6 ± 2.9 (0.60)	ND	ND	ND	0.11 ± 0.04 (0.73)	1.7 ± 3.0 (0.33)
	<i>rec102::URA3 spo13-1</i>								5.9 ± 1.5 (1.3)
C3-15	<i>rec102-1 SPO</i>	3.7 ± 0.4 (0.34)	2.4 ± 0.5 (0.46)	4.1 ± 1.4 (0.95)	2.5 ± 4.6 (1.3)	ND	ND	0.85 ± 14.6 ^b (5.7)	7.4 ± 11 (1.4)
	<i>rec102-1 SPO</i>								5.9 ± 1.3 (1.3)
RM162	<i>rec107-1 spo13-1</i>	ND	ND	6.2 ± 7.9 ^c (1.4)	2.6 ± 1.7 (1.4)	0.36 ± 0.18 (1.0)	0.25 ± 0.78 (0.83)	0.27 ± 0.16 (1.8)	2.0 ± 1.7 (0.38)
	<i>rec107-1 spo13-1</i>								6.7 ± 13 ^c (1.4)

^a Each 5-ml culture was grown from 100 cells per ml to approximately 2×10^7 cells per ml (range, 1×10^7 to 5×10^7 cells per ml), and a total of five independent cultures were examined for each diploid. The values given are the geometric means ± standard deviations. Values in parentheses are the recombination frequencies relative to the recombination frequency of the wild-type C3-16 strain. ND, not determined.

^b The large standard deviation is due to one of the five cultures having high ("jackpot") recombination frequencies due to the occurrence of a recombination event early in the growth of the culture.

^c Value from Malone et al. (23).

^d Value from Malone and Hoekstra (25).

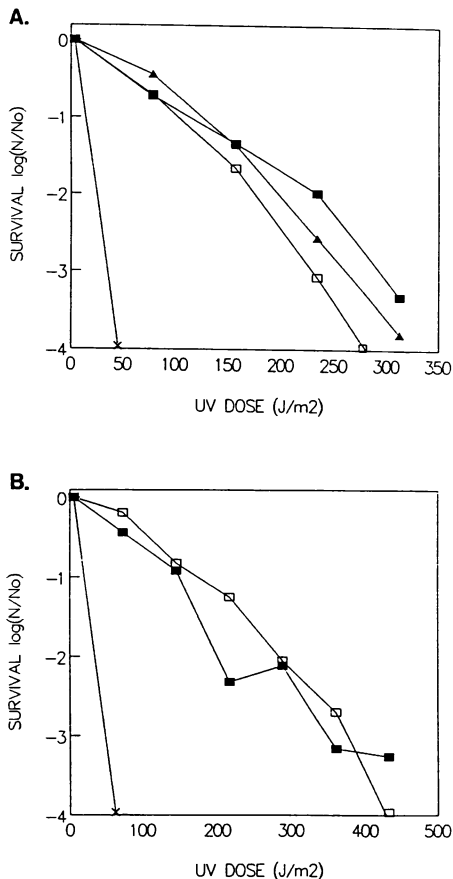


FIG. 1. (A) *REC102* and *REC107* are not required for UV resistance. Haploid strains that were wild type (Rec^+) (□) or contained the *rad1-2* (×), *rec102-1* (■), or *rec107-1* (▲) mutation were UV irradiated at 2.6 J/m²/s. Survival was calculated as follows: $\log(\text{concentration of surviving cells}/\text{initial number of cells per milliliter})$ [$\log(N/N_0)$]. (B) In a separate experiment (with different UV lamps), the UV sensitivity of a haploid containing the *rec102::URA3* null mutation (■) was determined as for panel A. Symbols for the other strains are as for panel A.

and *rec107* mutations reduce meiotic crossing-over. The average increases in meiotic recombination for all loci examined in this experiment are 1,500-fold for wild type, 0.8-fold for *rec102*, 0.91-fold for the null *rec102::URA3*, and 1.4-fold for *rec107*.

To verify that meiotic crossing-over was reduced in the presence of the *rec* mutations, we dissected dyad asci from *rec102-1 spo13-1* and *rec107-1 spo13-1* diploids. We examined crossing-over between the centromere and 10 loci on four different chromosomes (Table 4). The number of recombinant-type dyads is greatly reduced in both the *rec102-1* and the *rec107-1* strains; in fact, no recombinant dyads were observed in the *rec102-1* diploid. As was observed for *rad50* (24) and other early Rec^- mutations, both *rec102-1* and *rec107-1* reduce the frequency of aberrant-type dyads compared with the wild-type control. This is consistent with the interpretation that exchange is deleterious to an equational division (22, 24). Analysis of six intervals in 56 dyads from a *rec102::URA3* strain generated no recombinant dyads (data not shown).

Analysis of the cloned *REC102* and *REC107* genes. The selection scheme used to clone the wild-type *REC* genes by

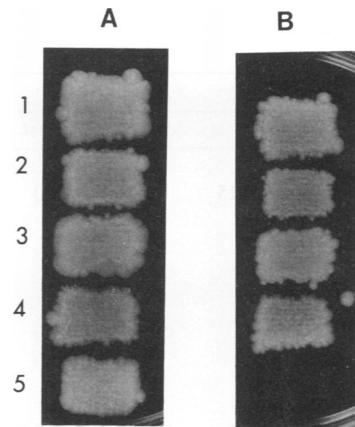


FIG. 2. *REC102* and *REC107* are not needed for methyl methanesulfonate resistance. Patches of the *rec102-1*, *rec102::URA3*, and *rec107-1* haploid strains were grown on YPD plates. Each plate was replica plated to another YPD plate (A) and a YPD plate containing 0.048% methyl methanesulfonate (B). Even though this concentration is almost five times more than that normally used (0.01%) to detect *rad* mutants, there was no indication of a growth deficiency in the Rec^- strains. Strains present: 1, wild-type haploid control; 2, *rec102-1* haploid; 3, *rec102::URA3* haploid; 4, *rec107-1* haploid; 5, *rad50-4* haploid.

complementation has been described previously (23). Subclones of the original isolates were constructed in an integrating vector and used to verify that the cloned DNA cosegregated with the appropriate *REC* locus (23). Figure 3 illustrates the restriction maps of fragments complementing the two Rec^- mutations and the ability of various subclones to complement the sporulation, recombination, and meiotic viability defects conferred by the *rec102-1* and *rec107-1* mutations. From these data, we inferred the location of the *REC* gene in the cloned DNA and proceeded to sequence this region (see Materials and Methods).

We noted the similarity of the restriction map for *REC107* to the published map for *MER2* (8). Analysis of the DNA fragment between the *EcoRI* and *PstI* restriction sites generated a sequence almost perfectly homologous to that published for *MER2* (11) (Fig. 4). The three differences found may be sequencing errors or may represent polymorphisms between the two strains from which the libraries were made. We confirmed that a *mer2::ADE2* mutation was unable to complement a *rec107-1* mutation for sporulation, spore viability, or meiotic recombination (data not shown). We conclude that the *REC107* and the *MER2* genes are identical.

The sequence of the *REC102* gene is shown in Fig. 5. Searches of the GenBank and EMBL data bases (6) revealed no homologies to any published gene. The sequence predicted a protein of 200 amino acids with a molecular weight of 23,240, a net charge of -2, and an isoelectric point of 6.52. The hydropathicity plot of the protein sequence is shown in Fig. 6; the plot is consistent with a soluble protein. Examination of the protein sequence demonstrated no zinc fingers, calcium binding, homeo box, or conserved kinase motifs (6). A consensus leucine zipper is found between bp 406 and 471 (21). This suggests that the native protein may be a dimer; alternatively, it may interact with other proteins to create heterodimers. This sequence does not appear to be part of a DNA-binding domain, as there is no upstream region of basic amino acids (21).

TABLE 3. Meiotic recombination in cultures of *spo13* diploids^a

Frequency type and locus	Recombination frequency ^b											
	Rec ⁺			<i>rec102-1</i>			<i>rec102::URA3</i>			<i>rec107-1</i>		
	Mitosis	Meiosis	Increase	Mitosis	Meiosis	Increase	Mitosis	Meiosis	Increase	Mitosis	Meiosis	Increase
Prototroph frequency												
<i>lys2-1</i>	0.15	300	2,000	0.15	0.19	1.3	0.11	0.05	0.45	0.27	0.40	1.5
<i>lys2-2</i>												
<i>tyr1-1</i>		ND			ND			ND		0.25	0.40	1.6
<i>tyr1-2</i>												
<i>his7-1</i>		ND			ND			ND		0.36	0.10	0.28
<i>his7-2</i>												
<i>ura3-1</i>	11	5,000	450	7.7	2.3	0.3		ND			ND	
<i>ura3-52</i>												
<i>met13-c</i>		ND			ND			ND		2.6	0.9	0.35
<i>met13-d</i>												
<i>trp5-c</i>	5.2	9,200	1,800	2.2	1.1	0.5	1.4	0.79	0.56		ND	
<i>trp5-2</i>												
<i>leu1-c</i>	4.3	6,200	1,400 ^c	4.7	1.4	0.30 ^c	2.6	2.5	0.96	6.2	3.4	0.55 ^c
<i>leu1-12</i>												
Drug-resistant-colony frequency												
<i>can1^r</i>	4.7	7,900	1,700 ^c	9.0	20	2.2 ^c	5.9	8.2	1.4	6.7	25	3.7 ^c
<i>CAN1^S</i>												
<i>cyh2^r</i>	5.2	9,600	1,800	8.4	1.5	0.18	1.7	2.0	1.2	2.0	4.0	2.0
<i>CYH2^S</i>												

^a Each diploid was sporulated, and the frequency of recombination was measured from three independent cultures for each diploid. The data presented are the geometric means of the three cultures.

^b The Rec⁺ diploid was C3-16, the *rec102-1* diploid was C3-13, the *rec102::URA3* diploid was C7-2, and the *rec107-1* diploid was RM162. Prototroph frequencies are to be multiplied by 10⁻⁵. Drug-resistant-colony frequencies are to be multiplied by 10⁻⁴. ND, not determined.

^c Value from Malone et al. (23).

Several meiotic genes that are expressed early in meiosis contain the URS1 sequence (5'-YCGGCGGCTA-3') (3). This sequence is usually located 100 to 200 bp upstream from the protein-coding region. We were surprised to find that no such sequence was found in *REC102*, at least as far upstream as -300 bp. However, examination of the *REC102* sequence indicates the presence of several DNA sequences closely corresponding to consensus binding sites for known transcription factors (Fig. 5) (see Discussion). Likewise, there is a consensus transcription termination region (39).

Map location of *REC102*. With the cloned *REC102* gene used as a probe, a Southern blot of a contour-clamped homogeneous electric field gel of yeast chromosomes revealed that *REC102* was located on either chromosome IV or chromosome XII (data not shown). The crosses described in Table 5 indicate that *REC102* is located 26.4 centimorgans (cM) from *CDC25* on chromosome XII. The data are consistent with a location between *CDC25* and *URA4*.

Analysis of *REC102* expression. Since mutations in *REC102* appeared to affect only meiosis, it seemed reasonable that it might be expressed only in meiotic cells. To test this hypothesis, a Northern analysis was done on RNA isolated from wild-type (*REC102 SPO13*) cells growing exponentially on glucose or acetate, a saturated glucose culture, and meiotic cells. As shown in Fig. 7, *REC102* mRNA from exponentially growing cells (in glucose or acetate) or from stationary-phase cells in glucose is not visible, consistent with the lack of a mitotic phenotype for the *rec102* mutations. In contrast, expression begins after 2 h in sporulation medium and appears to peak at 4 h, about the time in meiosis when recombination is beginning. We noted that the 1.79-kb (small) rRNA and 3.39-kb (large) rRNA hybridize under the conditions used in this experiment (this phenomenon has

been observed before [37]). To quantitate the amount of *REC102* mRNA in mitosis or in meiosis, densitometry of the Northern analyses was done, and the amount of *REC102* mRNA relative to *PYK1*, the large rRNA, and the small rRNA was calculated (Fig. 8A). All the measurements indicate that the peak of expression occurs at 4 h into meiosis and that expression decreases after this time.

To correlate the time of mRNA expression with other events in meiosis, *REC102 SPO13* cells were sampled for the timing of other meiotic events. As shown in Fig. 8B, recombinants begin to increase at about 3 h, and the first immature asci were observed at 10 h. We have observed that *rec102::URA3 SPO13* cells display no increase in meiotic recombination, but they do enter meiosis, and a majority of the cells proceed through the first division (4).

DISCUSSION

The initial data published by Malone et al. (23) suggested that the *REC102* and *REC107* genes were meiosis-specific recombination genes. The data presented here confirm this. In the presence of either a *rec102* or a *rec107* mutation, no increases are detected either in gene conversion (as measured at several heteroallelic loci on several chromosomes) or in crossing-over (as measured in several intervals on several chromosomes). We note that the phenotype of the null allele is identical to that of the first mutant allele isolated, *rec102-1*. From all of these observations, we conclude that both the *REC102* and the *REC107* genes are absolutely and generally required for meiotic recombination.

The data presented here also clearly indicate that mutations in the *REC102* and *REC107* genes do not affect spontaneous mitotic recombination. Furthermore, mutations in

TABLE 4. Analysis of meiotic crossing-over in *spo13* strains^a

Strain ^b	No. of dyads (P:R:A) with indicated mutation on chromosome ^c									
	VII					V				
	<i>mel13</i>	<i>cyh2</i>	<i>trp5</i>	<i>leu1</i>	<i>can1</i>	<i>wra3</i>	<i>lys2</i>	<i>gpr1</i>	<i>hst7</i>	III (<i>MAT1</i>)
C3-16 (<i>REC</i>)	ND	50:18:13 (22)	56:17:8 (21)	63:9:9 (11)	53:22:4 (28)	61:13:7 (16)	36:22:15 (30)	ND	29:20:7 ^d (36)	59:13:15 (15)
C3-14 (<i>rec102-1</i>)	ND	41:0:1 (<2)	148:0:2 (<0.7)	148:0:2 (<0.7)	93:0:2 (<1)	146:0:4 (<0.7)	146:0:4 (<0.7)	ND	147:0:3 (<0.7)	158:0:4 (<0.6)
RM162 (<i>rec107-1</i>)	160:0:2 (<0.6)	160:1:1 (0.6)	ND	162:0:0 (<0.6)	160:1:2 (0.6)	ND	160:1:1 (0.6)	160:1:1 (0.6)	160:1:1 (0.6)	159:0:3 (<0.6)

^a Diploids were sporulated and dissected, and dyads having two viable spores were analyzed.

^b The recombination genotype of the diploid is shown in parentheses.

^c Numbers in parentheses refer to the percent recombinant-type dyads. In cases in which no recombinant-type dyads were found, the percent recombinant-type dyads was calculated to be less than (1/total dyads) × 100. P, parental-type dyads; R, recombinant-type dyads; A, aberrant-type dyads (22).

^d Data from Malone (22).

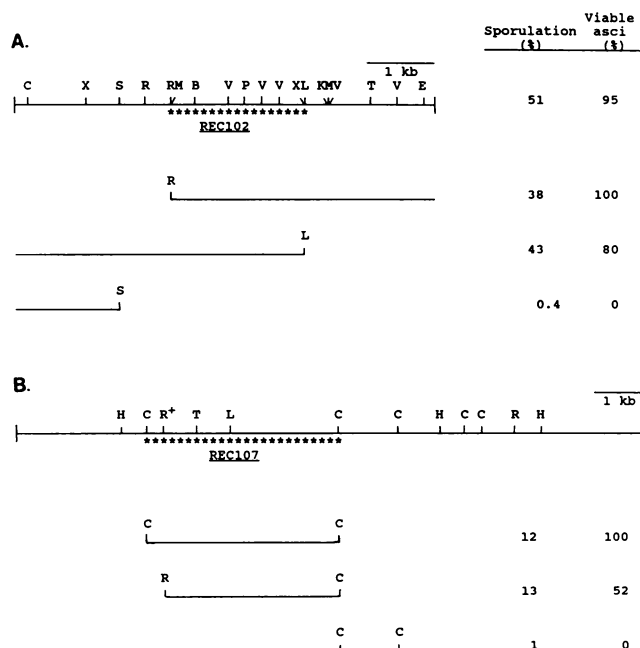


FIG. 3. Restriction maps of clones containing *REC102* (A) and *REC107* (B). The original 7-kb cloned DNA fragment (pCM201) complemented the *rec102-1* mutant; likewise, the original 15-kb cloned fragment (pCM701) complemented *rec107-1*. Subclone maps are shown beneath the map of the original clone. The abilities of the various subclones to complement the *rec* mutation indicated that the genes were located in the regions denoted by asterisks. The transformed diploid was sporulated, and the viability of the asci was examined. B, *Bsp*El; C, *Cl*aI; H, *Hind*III; E, *Spe*I; K, *Kpn*I; L, *Sal*I; M, *Bam*HI; P, *Pvu*I; R, *Eco*RI; S, *Sst*I; T, *Pst*I; V, *Eco*RV; X, *Xho*I.

either gene do not affect mitotic DNA repair of UV or methyl methanesulfonate damage. Our previous work showed that the mutations had no effect on mitotic growth rate (23). We infer from the lack of mitotic phenotypes that the *REC102* and *REC107* genes are probably not necessary in mitosis.

In the course of our study of the cloned *REC107* gene, we found that the restriction map resembled the published map for the *MER2* gene, a gene also reported to be required for meiotic recombination (8). All the data indicate that the *REC107* and the *MER2* genes are identical; we will henceforth refer to the *REC107* gene as *MER2*. Engebrecht et al. (8) examined the effect of a *mer2::ADE2* mutation on meiotic recombination at only one heteroallelic locus; although no mitotic value was given, the meiotic frequency was reduced



FIG. 4. *REC107* and *MER2* are the same gene. A sequence of the complementing clone for *REC107* was obtained starting at the *Eco*RI site indicated in Fig. 3B by R⁺ and extending for 162 bp to the right as drawn. This sequence was compared with that of *MER2* (11). The numbering is as described previously for *MER2* (11).

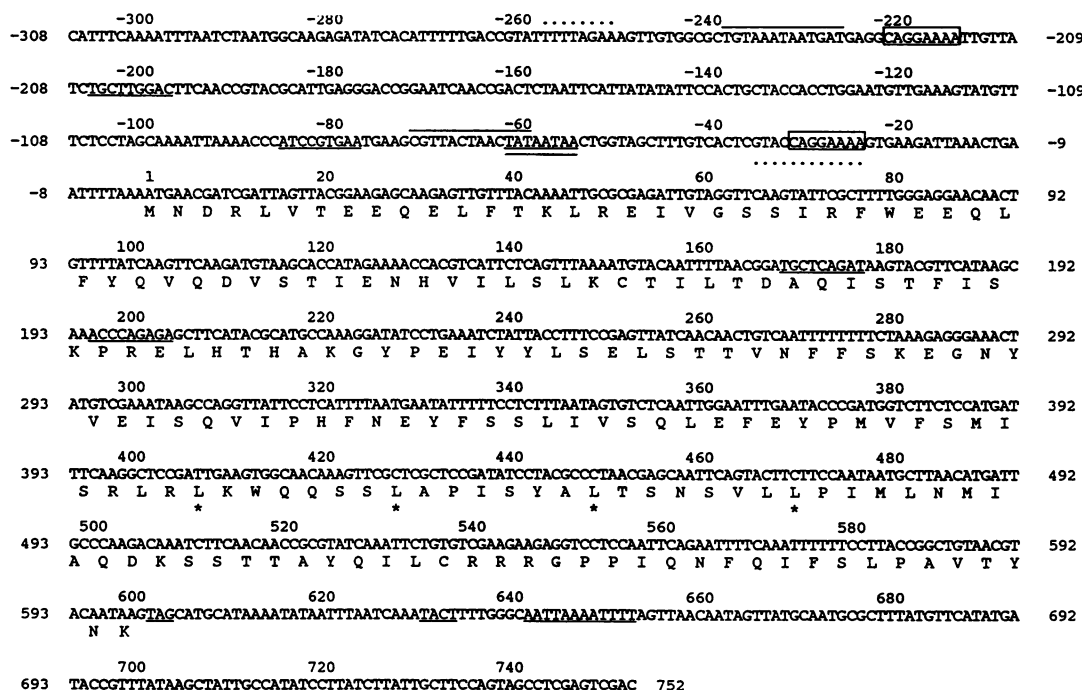


FIG. 5. Nucleotide sequence of the *REC102* gene. The presumed open reading frame begins at position 1, and a termination codon is situated at position 600, resulting in a protein of 200 amino acids. A TATA box is tentatively located at the double-underlined region (–62) in the putative promoter region of the *REC102* gene. A consensus poly(A)⁺ addition site is underlined at the 3' end of the sequence (+630 and +641) (39). Potential RAP1 binding sites are underlined at positions –206, –86, +168, and +195. The binding sites at positions –206 and +168 are the complements of the consensus sequence. Potential ABF1 binding sites are designated by lines above the sequence at positions –239 and –72. Both sequences shown are the complements of the published consensus sequence. Two potential SW14.6 binding sites are boxed at positions –222 and –32. A perfect consensus cell cycle box is located at position –36 and is indicated by a dotted line underneath the sequence. Finally, a possible heat shock transcription factor binding sequence is shown at position –258 and indicated by a dotted line above the sequence. Consensus sequences were determined from reference 38. A consensus leucine zipper sequence is indicated by asterisks under the appropriate leucines at positions 406 to 471.

200-fold compared with that of wild-type cells. Our data confirm that meiotic induction of recombination requires *MER2* and support the proposal presented by Engebrecht et al. (8) that *MER2* is a general meiotic Rec gene.

The *REC102* gene is not detectably expressed in mitotic cells, consistent with its proposed role as a meiosis-specific gene. Neither the elimination of glucose nor the entry into stationary phase allows mitotic expression. However, mRNA is detected early in meiosis and peaks at approximately the time of recombination initiation (4 h). The persistence of *REC102* mRNA may reflect the asynchrony of the culture, although most of the recombination appears to be completed by 12 h. The expression pattern suggests that there are at least three levels of controls for expression of *REC102*. First, there must be a method of negative control to keep the gene turned off in mitotic cells; second, there must

be a positive control to turn it on at precisely the appropriate time in meiosis; and third, there appears to be a negative control which reduces expression later in meiosis. It is possible that the control mechanism for the reduction in mRNA after 6 h in meiosis is the same as the control that negatively regulates expression in mitotic cells.

Analysis of published studies of meiotic gene expression indicates that other genes involved in chromosome pairing and recombination show an mRNA expression pattern similar to that of *REC102* (in the sense of turning on early and turning down or off after a few hours) (e.g., *RED1* [37] and *SPO13* [3]). It is interesting that genes showing this pattern can affect three (potentially) different, but interrelated, processes: *RED1* may be required primarily for pairing, *REC102* is required for early recombination, and *SPO13* is required for the proper reductional division. Buckingham et al. (3)

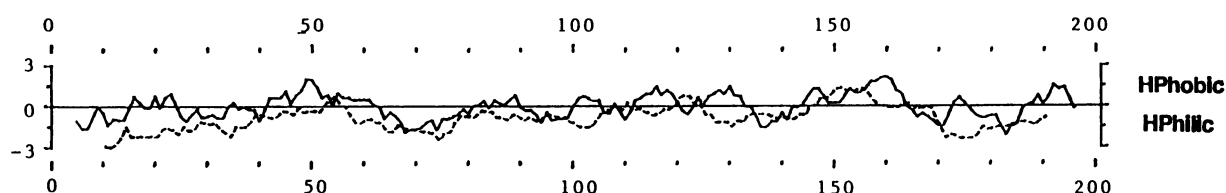


FIG. 6. Hydrophobicity plot of the deduced *REC102* protein sequence with 200 amino acids. The solid line is based on the Kyle-Doolittle algorithm, and the dotted line is based on the Goldman algorithm (6).

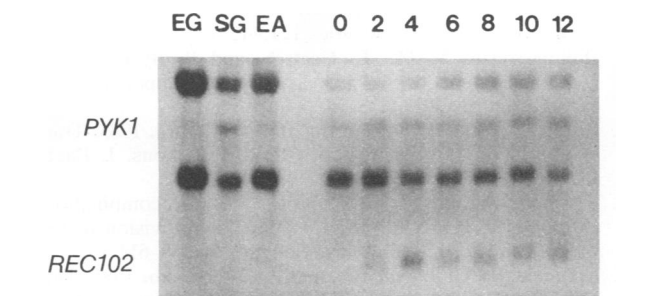


FIG. 7. *REC102* is expressed only during meiosis. Total RNA was isolated from a *Rec*⁺ yeast diploid after growth in YPD or YPA and at various times after transfer to sporulation media. The RNA was analyzed by the standard Northern procedure (32). To monitor expression of *REC102*, a riboprobe (Stratagene) from the region +10 to +538 was used. To verify the presence of RNA in each lane, a DNA probe corresponding to the coding region of the *PYK1* gene was used (see Materials and Methods). Cross-hybridization of the 3.39- and 1.79-kb rRNAs occurred (see the text). Lanes: EG, RNA from cells in YPD during exponential growth; SG, RNA from cells in YPD in stationary phase; EA, RNA from cells in YPA during exponential growth. Numbers from 0 to 12 refer to the time (in hours) after transfer to sporulation media.

examined the promoter regions of several genes required in meiotic I prophase and found that the only sequence common to *SPO13*, *SPO11*, *SPO16*, *HOP1*, *RED1*, and *MER1* was a URS1 consensus sequence (5'-YCGGCGGCTA-3')

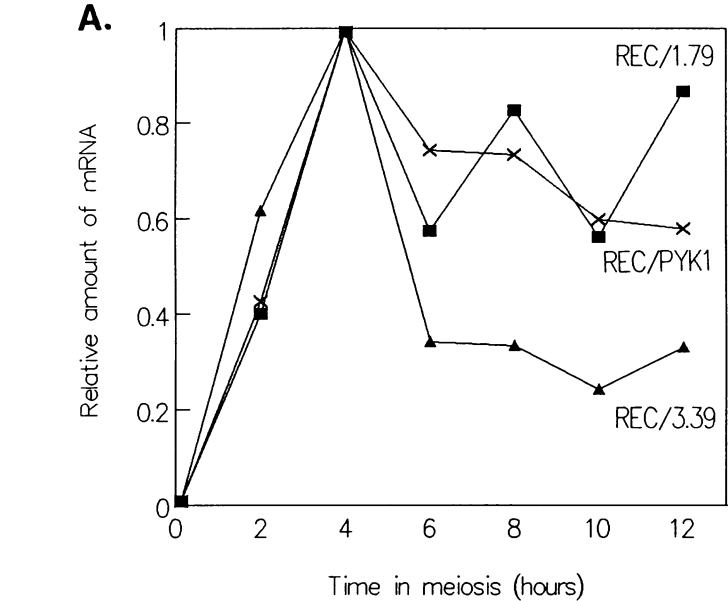


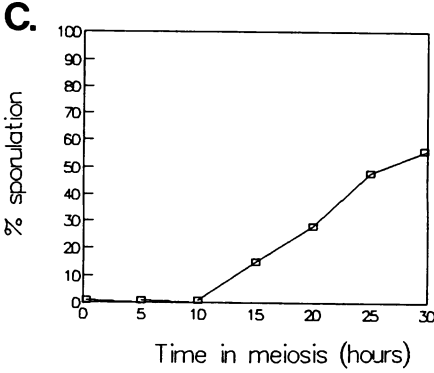
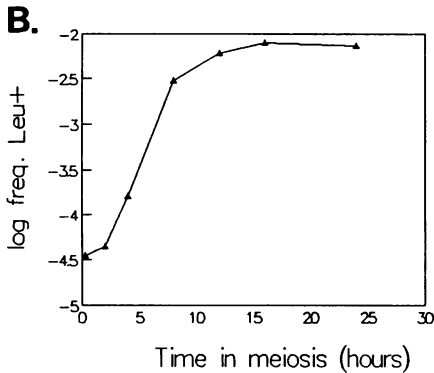
FIG. 8. *REC102* mRNA in meiosis in a *REC102 SPO13* diploid. (A) Relative amounts of *REC102* RNA present during meiosis (see Fig. 7 and Materials and Methods). The highest ratio was normalized to 1; in all three cases this occurred at 4 h. (B) Kinetics of meiotic intragenic recombination measured by the frequency of *Leu*⁺ prototrophs (from *leu1-c/leu1-12* heteroalleles). (C) Kinetics of sporulation.

TABLE 5. Meiotic mapping of *REC102*

Chromosome XII cross	No. of tetrads with marker:			Map distance (cM) ^a
	P	N	T	
<i>rec102</i> × <i>ura4</i>	9	2	12	58.1
<i>rec102</i> × <i>cdc25</i>	39	1	32	26.4

^a Map distances were calculated by the formula of Perkins (28): cM = 100(T + 6N)/2(P + N + T). For map distances greater than 35 cM, the empirical correction described by Sherman and Waken (35) was used. P, parental ditype; N, nonparental ditype; T, tetratype.

usually located about 100 to 200 bp upstream from the AUG codon. This sequence is not found in *REC102* (Fig. 5), either in the coding region or as far as 300 bp upstream from the first ATG codon. We do note a number of sequences that could be involved in regulation of *REC102* (Fig. 5). There are four potential RAP1 binding sites, two possible ABF1 binding sites, two potential SWI4.6 binding sites, a possible cell cycle box, and a heat shock factor binding sequence (38) (Fig. 5). The sequences present in *REC102* differ from the published consensus by one base in all cases except for the cell cycle box, which matches the consensus perfectly (38). This multitude of regulatory sequences would potentially allow a variety of combinatorial controls. Several of these sequences are located at positions typical of known *cis*-acting transcriptional control regions. Note that two of the



putative RAP1 binding sites are located in the coding region. Control sequences in the coding region of meiotic genes have been reported previously; the *SPO11* gene contains a URS1 consensus sequence at position +163 (3). The clustering of several different types of transcriptional control sequences in the *REC102* gene raises the possibility of interactions between the transcription binding factors. We are in the process of determining which of these sequences are important in the meiosis-specific expression of *REC102*.

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